DOI:
10.1002/art.41156

Document Version
Peer reviewed version

Synovial IL-17A+ CD8+ T cells display a polyfunctional, pro-inflammatory and tissue-resident memory phenotype and function in psoriatic arthritis

Kathryn J.A. Steel (PhD)[1], Ushani Srenathan (PhD)[1*], Michael Ridley (PhD)[1*], Lucy E. Durham (MBBS)[1], Shih-Ying Wu (MSc)[1], Sarah E. Ryan (MRes)[1], Catherine D. Hughes (MB BCh BAO, MSc, MRCP)[1,2], Estee Chan (BHB, MBChB, FRACP)[2], Bruce W. Kirkham (MD, FRCP, FRACP)[2*], Leonie S. Taams (PhD)[1*]

[1] Centre for Inflammation Biology and Cancer Immunology (CIBCI), Department of Inflammation Biology, School of Immunology & Microbial Sciences, King's College London, [2] Department Rheumatology, Guy's & St Thomas' Hospital, London, UK

*US and MR contributed equally
*BWK and LST contributed equally

Corresponding author: Prof Leonie Taams, Centre for Inflammation Biology and Cancer Immunology (CIBCI), Department of Inflammation Biology, School of Immunology & Microbial Sciences, King's College London, 1st floor New Hunt's House, Guy's Campus, London SE1 1UL, UK.
Telephone: +44 20 7848 8633
Email: leonie.taams@kcl.ac.uk
ORCID: 0000-0002-9337-7194

Conflict of interest statement: This study was supported in part by research support from Novartis Pharma AG. LST has received speaker fees and/or research support from GSK, Novo Nordisk A/S, UCB and Novartis. BWK has received research support from Eli Lilly, Novartis, Roche Pharmaceuticals and UCB Pharma. He has been an advisor or received speaker fees from Eli-Lilly, Janssen, and Novartis. The authors have no additional financial interests to declare.

ABSTRACT

Objective: Genetic associations imply a role for CD8+ T cells and the IL-23/IL-17 axis in psoriatic arthritis (PsA) and other spondyloarthritides (SpA). IL-17A+CD8+ (Tc17) T cells are enriched in the synovial fluid of patients with PsA and IL-17A blockade is clinically efficacious in PsA/SpA. Our aim was to determine the immunophenotype, molecular profile and function of synovial Tc17 cells in order to elucidate their role in PsA/SpA pathogenesis.

Methods: Peripheral blood (PB) and synovial fluid (SF) mononuclear cells were isolated from patients with PsA/SpA. Cells were phenotypically, transcriptionally and functionally analysed by flow cytometry (n=6-18), TCRβ sequencing (n=3), RNA-seq (n=3), RT-qPCR (n=4) and Luminex/ELISA (n=4-16).

Results: IL-17A+CD8+TCRαβ+ T cells were increased in the SF vs. PB of patients with established PsA ($p<0.0001$) or other SpA ($p=0.0009$). TCRβ sequencing showed these cells are polyclonal in PsA (median clonality = 0.08), whilst RNA-seq and deep-immunophenotyping revealed that PsA synovial Tc17 cells have hallmarks of Th17 (RORC/IL23R/CCR6/CD161) and Tc1 cells (granzyme A/B). Synovial Tc17 cells showed a strong tissue-resident memory T cell signature and secreted a range of pro-inflammatory cytokines. We identified CXCR6 as a marker for synovial Tc17 cells, and increased levels of CXCR6 ligand CXCL16 levels in PsA SF ($p=0.0005$), which may contribute to their retention in the joint.

Conclusion: Our results identify synovial Tc17 cells as a polyclonal subset of tissue-resident memory T cells characterised by polyfunctional, pro-inflammatory mediator production and CXCR6 expression. The molecular signature and functional profiling of these cells may help explain how Tc17 cells can contribute to synovial inflammation and disease persistence in PsA and possibly other SpA.

INTRODUCTION

Psoriatic arthritis (PsA) is part of an umbrella group of inflammatory diseases, termed spondyloarthritis (SpA), which share common patterns of joint inflammation (peripheral and axial), skin, gut and eye manifestations, genetic components, and the absence of diagnostic autoantibodies (seronegative). In addition to psoriatic arthritis (PsA), SpA includes ankylosing spondylitis/non-radiographic axial spondylitis (AS/NRAS), reactive arthritis (ReA), enteropathic arthritis (EA) and undifferentiated SpA, with a combined prevalence of 1-2% (1).

It is increasingly recognised that the IL-23/IL-17 pathway plays a major role in PsA/SpA immunopathogenesis (2, 3). Therapies targeting IL-17A show clinical efficacy in patients with PsA and AS (4, 5), whilst several genetic loci implicated in the IL-17/IL-23 axis including *IL12B* (IL-12p40), *IL23R* and *TRAF3IP2* (Act1) are associated with PsA and AS susceptibility (6, 7). To date, the majority of studies have focused on identifying IL-17A producing CD4+ T (Th17) cells or innate lymphoid type 3 (ILC3) cells in the inflamed joints of patients with PsA/SpA, yet the strong association of MHC class I and other CD8+ T cell/MHC class I related loci (*RUNX3, ERAP1/2*) suggests that CD8+ T cells play an important role in PsA/SpA (7-9). We previously demonstrated the enrichment of IL-17A-expressing CD8+ T (Tc17) cells in the synovial fluid of patients with PsA (10); Tc17 cells have also been reported in the synovial fluid of patients with juvenile idiopathic arthritis (11) and at the site of inflammation in other immune-mediated inflammatory diseases (reviewed in (2, 12)). Recent murine models and transcriptional analysis of healthy human spleen Tc17 cells have shed light onto the function of Tc17 cells (13, 14). However, functional and molecular analysis of human synovial Tc17 cells is essential to elucidate the role of synovial Tc17 cells in PsA/SpA pathogenesis.

The enrichment of Tc17 in the inflamed joint raises the question as to whether these cells migrate into the joint or are persistently present. Recently, a novel subset of CD8+ effector T cells enriched in tissue compartments without significant presence in the blood has been described (15). These tissue resident memory (T_{RM}) T cells are characterised by expression of CD69 and CD103, defined by a core transcriptional signature (16) and possess the potential to produce pro-inflammatory

cytokines including IL-17A, IL-22 and IFN-γ, as well as granzymes and perforin (reviewed in (17, 18)). The presence of T_{RM} cells in humans has been described in skin, lung, gut and brain tissue and a recent study reported the presence of CD8+ T cells with a tissue-resident memory phenotype in the synovial fluid of JIA patients (11). As such, T_{RM} cells are hypothesised to contribute to the immunopathogenesis of human immune-mediated inflammatory disease. However, if and how Tc17 and T_{RM} cells relate to each other is not well established.

To enhance our understanding of the function and molecular biology of human IL-17A+ CD8+ T cells, we performed extensive phenotypic, molecular and functional profiling of human Tc17 cells derived from the PsA synovial fluid. Using flow cytometry, TCR sequencing, Luminex and RNA-seq analysis, we demonstrate that PsA synovial IL-17A+ CD8+ T cells have a polyclonal TCR repertoire, a polyfunctional, pro-inflammatory cytokine profile, and many hallmarks of T_{RM} cells. These features position Tc17 cells as relevant contributors to the initiation or perpetuation of chronic inflammation in PsA, and possibly other SpA or IL-17A/HLA class I-associated inflammatory diseases.

MATERIALS AND METHODS

Study subjects

Peripheral blood and synovial fluid samples were obtained from patients with psoriatic arthritis or other peripheral SpA (ankylosing spondylitis/non-radiographic axial-SpA, reactive arthritis, enteropathic arthritis) attending the Rheumatology Department, Guy's Hospital. Patients fulfilled the CASPAR or 2010 ACR/EULAR criteria (19, 20). Demographic and clinical characteristics of patients are shown in Supplementary Table 1. All subjects provided written informed consent. Ethical approval was obtained from Bromley Research Ethics Committee (06/Q0705/20) and Harrow Research Ethics Committee (17/LO/1940).

Cell isolation

Mononuclear cells (PBMC and SFMC) were isolated using Lymphoprep™ (Axis-Shield, Oslo, Norway) and washed in culture medium (RPMI 1640 supplemented with 10% FSC + 1% penicillin, streptomycin, L-glutamine). Cells were cryopreserved and stored in liquid nitrogen in culture medium supplemented with 50% FCS and 10% dimethyl sulfoxide (all Thermo-Fisher, Waltham, MA, USA).

Flow cytometry

Thawed cells were rested for 1 hr at 37°C, 5% CO_2. For intracellular staining samples were stimulated with phorbol myristate acetate (PMA, 50 ng/ml) and ionomycin (750 ng/ml, both Sigma-Aldrich, St Louis, MO, USA) in the presence of Golgistop (BD Biosciences, Franklin Lakes, NJ, USA) for 3 hours at 37°C, 5% CO_2. Cells were stained with eFluor780 Viability Dye (eBioscience, San Diego, CA, USA) and surface staining was performed at 4°C. Cells were fixed with 2% PFA and permeabilised using 0.5% Saponin (Sigma-Aldrich). Antibodies are described in Table S2. Samples were acquired using an LSR-Fortessa (BD Biosciences). Data were analysed using FlowJo (v10, Tree Star, Ashland, OR).

TCRβ sequencing

Extracted DNA (Qiagen, Venlo, Netherlands) underwent bias-controlled amplification of VDJ rearrangements followed by high throughput sequencing (ImmunoSEQ, Adaptive Biotech, Seattle, WA, USA). Data from productive reads (sequence level)

were analysed using the ImmunoSEQ analysis platform (Adaptive Biotech). Clonality was defined as 1-Pielou's Evenness and ranged from 0 (indicating a highly polyclonal repertoire) to 1 (indicating a monoclonal repertoire). Overlap was determined using Morisita's index ranging from 0 (indicating no similarity between two populations) to 1 (indicating complete similarity between two populations).

Cell sorting

For T_{RM} T cell sorting, SFMC were stained with eFluor780 and CD3, CD4, CD8, CD14, CD69 and CD103 antibodies (Supplementary Table 2). Post sort, CD8+ T_{RM} subsets were stimulated, fixed and permeabilised before intracellular cytokine staining for IL-17A and IFN-γ. For sorting of cytokine producing cells, magnetically isolated (Miltenyi Biotec, Bergisch Gladbach, Germany) CD3+ T cells were stimulated for 1.5 hours at 37°C with PMA (50 ng/ml) and ionomycin (750 ng/ml) before staining using an IL-17A and, where indicated, IFN-γ cytokine secretion assay (Miltenyi). To identify cytokine-producing T cell subsets, cells were counter stained with eFluor780 and anti-CD3, CD8, CD14 and CD4 antibodies. Cells were sorted using a BD FACS Aria and acquired using an LSR-Fortessa (BD Biosciences).

RNA sequencing and RT-qPCR

Libraries were prepared by GENEWIZ LTD (South Plainfield, NJ, USA) and sequenced on the HiSEQ2500 platform (Illumina, San Diego, CA, USA) at a depth of 28-40M reads. Low quality (phred score <20) bases and adapters were trimmed using TrimGalore! and reads aligned (hg38) using RNA STAR. Paired reads were quantified using FeatureCounts. PCA analysis and statistical comparison of gene expression was performed using DESeq2.

For RT-qPCR, total RNA was extracted from sorted cell subsets (Qiagen). cDNA was generated using the high capacity cDNA reverse transcription kit (Thermo-Fisher). RT-PCR was performed using the SensiFAST SYBR kit (Bioline, Cincinnati, OH, USA) and primers from Integrated DNA Technologies (Coralville, IA, USA) listed in Supplementary Table 3.

Luminex assay and CXCL16 ELISA

Supernatants were obtained from sorted T cell subsets cultured for 24-hours in culture media. A custom magnetic Luminex (Bio-Techne, Minneapolis, MN, United States) was analysed on the Luminex FlexMap 3D platform (Austin, TX, United States). Serum and synovial fluid were analysed using a CXCL16 ELISA (Bio-Techne).

Graphics and statistical analysis

Statistical analysis and graphical illustration was performed using either PRISM (v7, GraphPad, San Diego, CA, US), or ggplot2 using R version 3.5.2. Results are expressed as the median + interquartile range (IQR). Wilcoxon's matched pairs signed rank test or Friedman multiple comparisons test were used.

RESULTS

Tc17 cells are polyclonal TCRαβ+ memory cells enriched in PsA/SpA joints

To determine whether synovial Tc17 are enriched only in PsA, or also in other SpA, we stimulated paired PBMC and SFMC from patients with PsA, other SpA types or RA *ex vivo* with PMA/ionomycin and assessed the frequency of IL-17A+ CD8+ T cells by flow cytometry (Figure 1A, B; gating strategy in Supplementary Figure S1). Low frequencies of Tc17 cells were detected in PBMC from PsA, other SpA and RA patients (median 0.1%), with no significant differences observed between these groups. The frequencies of Tc17 cells were significantly increased in SFMC compared to PBMC in patients with PsA ($p<0.0001$) and other SpA types ($p=0.0009$), but not RA ($p=0.16$). The frequencies of Th17 cells were similar between the three disease groups (Supplementary Figure S1B).

Synovial Tc17 cells were predominantly comprised of TCR$\alpha\beta$+ cells, with small proportions of MAIT (Vα7.2+), $\gamma\delta$ and NKT cells (Figure 1C, D). Over 98% of synovial Tc17 cells exhibited a memory phenotype (CD45RA-CD27+/-; Figure 1E, F), whilst a considerable proportion of Tc17 cells expressed the immunoinhibitory receptor programmed cell death protein 1 (PD1; median 65%) and activation marker HLA-DR (51%), suggesting these cells previously experienced antigen stimulation (Figure 1G, H).

The presence of clonally restricted memory CD8+ T cells with features of antigen-specific expansion has been described in the synovial fluid, synovial tissue and skin of patients with PsA (21-23). To investigate whether in PsA, synovial Tc17 cells are also clonally restricted, bulk memory (CD45RA-CD27+/- and CD45RA+CD27-), IL-17A+IFN-γ+/- (Tc17) and IL-17A-IFN-γ+ (Tc1) memory CD8+ T cells were sorted from the SF of PsA patients (gating strategy in Supplementary Figure S2). TCRβ sequencing revealed that synovial Tc17 cells display a diverse TCR repertoire (Figure 2A) with a low clonality score (Pielou evenness), which was similar to synovial Tc1 and bulk memory CD8+ T cells (Figure 2A, B). For two patients, a substantial proportion of the clones found in synovial Tc17 cells were also present in the synovial Tc1 population, resulting in a Morisita overlap index >0.9 (Figure 2C, D).

The third patient had a lower number of productive templates, which may have resulted in a low Morisita score.

Molecular analysis of synovial Tc17 cells reveals commonalities in transcriptional profile with Th17 and Tc1 cells

Recent transcriptional analysis of healthy human spleen Tc17 cells revealed a distinct molecular profile compared to IL-17- CD8+ T cells or Th17 cells (14). To determine the molecular profile of Tc17 cells from the inflamed PsA joint, we sorted highly pure Tc17, Tc1 and Th17 cells from the SFMC of patients with PsA for RNA sequencing (gating strategy Supplementary Figure S3). Accurate sorting was confirmed by the normalised gene counts of *IL17A* and *IFNG* for each of the populations (Supplementary Figure S3B). Principal component analysis showed a degree of gene expression heterogeneity between synovial Tc17 cells from different patients. In 2/3 patients, the synovial Tc17 cells clustered separately from the Th17 cells but close to the Tc1 cells (Figure 3A, B), suggesting a proportion of the transcriptional profile between synovial Tc17 and Tc1 cells is shared. In total, 80 genes were differentially expressed between the synovial Tc17 and Tc1 subsets ($\leq$1%FDR, fold change >2; MA plots shown in Figure 3C with the ten most up- or down regulated genes indicated). Several genes relating to a type 17 response *(IL17A, IL17F, RORC, IL23R, CCR6* and *KLRB1)* were elevated compared to synovial Tc1 cells (Figure 3C, E). When synovial Tc17 cells were compared to synovial Th17 cells, 145 genes were found to be differentially expressed (Figure 3D). Upregulated genes in synovial Tc17 cells compared to Th17 cells included *CD8A and CD8B*, confirming our gating strategy, plus genes associated with cytolytic activity (*GRZA, GRZB and PRF1*) (Figure 3D, F). Expression levels of *TCF7* (encoding TCF-1), recently identified as a transcriptional regulator of mouse Tc17 cells through repression of MAF and RORγt (14) was low in synovial Tc17 cells (Supplementary Figure S3C).

We confirmed the transcriptional data for the type 17 markers chemokine receptor 6 (CCR6) and Killer cell lectin-like receptor subfamily B member 1 (CD161) at the protein level by flow cytometry. A high frequency of synovial Tc17 cells co-expressed

CCR6 or CD161, at levels comparable to synovial Th17 cells. In contrast, only limited proportions of synovial IL-17A- CD8+ or IFN-γ+ CD8+ T cells expressed these molecules (Figure 4A, B). viSNE analysis showed that CCR6 and CD161 were co-expressed by Tc17 cells (Figure 4C, representative of n=7). Notably, this analysis also revealed that CCR6 and CD161 expression is not restricted to the IL-17A+ population, i.e. these markers do not exclusively define IL-17A expression in synovial CD8+ T cells. RT-qPCR analysis further confirmed that transcript levels of retinoic acid receptor-related orphan receptor C (*RORC*) and interleukin 23 receptor (*IL23R*) in the synovial Tc17 population were comparable to Th17 cells, and higher than in IL-17A- CD8+ T cells (which contains IFN-γ+ CD8+ T cells) (Figure 4D).

Frequencies of granzyme A, granzyme B, CD107a and perforin expressing cells within Tc17 cells were variable but enhanced compared to synovial Th17 cells, whilst they were comparable to those within synovial Tc1 cells (Figure 4E, F). It should be noted however that limited expression of perforin and LAMP1/CD107a was observed in synovial Tc17 cells, suggesting these cells may not have full cytotoxic capability.

Tc17 cells have hallmarks of tissue-resident memory T cells

The observation that Tc17 cells with a memory phenotype are enriched in the PsA joint but not the blood prompted us to investigate whether synovial Tc17 cells from the inflamed joint expressed markers of tissue residency. Our molecular profiling revealed that genes reported to be transcriptional hallmarks of tissue resident memory (T_{RM}) cells (e.g. *ITGAE* (encoding CD103), *ZNF683* (encoding HOBIT), *CRTAM* and low levels of *S1PR1*) (16) were differentially expressed between synovial Tc17 and Th17 cells (Figure 5A). We confirmed the expression of the T_{RM} cell marker CD103 (αE integrin) on a high proportion of joint-derived Tc17 cells by flow cytometry (Figure 5B, C). CD69, which is also commonly used as T_{RM} marker, is upregulated upon PMA/ionomycin stimulation and was therefore not determined. Since Tc17 cells have been identified in the gut and the skin (24-27), sites that can also be affected in PsA/SpA, we assessed markers for the gut-related molecule integrin β7 (which is normally co-expressed with αE integrin) and the skin-related adhesion/homing molecules, cutaneous lymphocyte antigen (CLA) and CD49a (very

late antigen; VLA1). Considerable proportions of synovial Tc17 cells co-expressed integrin β7, CD49a/VLA-1 and to a lesser extent CLA (Figure 5B, C).

Based on these findings, we examined the presence of T_{RM} cells using the markers CD69 and/or CD103 in unstimulated SFMC from PsA patients (Figure 5D, E). Within the CD8+ T cell population, on average 11% of cells were CD69+CD103+ (range 2-16%), 26% of cells were CD69+CD103- (range 3.2-54%), and 6% of cells were CD69-CD103+ (range 1.29-16%). In the CD4+ compartment, 26% of the cells were CD69+CD103- whilst CD103 expression was negligible (expressed by <1% of CD4+ T cells). These data indicate that CD103+ T_{RM} cells in the inflamed PsA joint typically are CD8+ T cells.

To directly demonstrate that CD8+ T_{RM} cells contain IL-17A producing cells, we sorted synovial CD8+ T cells from PsA patients into highly pure CD69+CD103+, CD69+CD103-, CD69-CD103+ and CD69-CD103- subsets (gating strategy shown in Supplementary Figure S4). The sorted cells were then stimulated *ex vivo* and stained for IL-17A and IFN-γ (Figure 5F, G). After normalising for total cytokine expression, we identified that the CD69+CD103+ CD8+ T cell population contained the highest frequency of IL-17A+ cells, followed by CD69+ or CD103+ single expressing cells, whilst CD69-CD103- CD8+ T cells contained minimal IL-17A+ cells (Figure 5H). In contrast, frequencies of IFN-γ+ cells were distributed more equally amongst the 4 sorted cell subsets. We also found a significant correlation between the presence of IL-17A+ CD8+ T cells and CD69+CD103+ T_{RM} cells in the synovial fluid, further supporting a relationship between these two cell populations (Supplementary Figure S5). Together, these data indicate that synovial T_{RM} cells are enriched for IL-17A expression and that Tc17 cells form part of the tissue-resident memory T cell pool in the inflamed synovial joints in PsA.

Synovial Tc17 cells are polyfunctional inflammatory cells characterised by high expression of CXCR6

As a final step we sought to determine the functional potential of synovial Tc17 cells. For this, we assessed the co-expression and secretion of pro- and anti-inflammatory

cytokines by flow cytometry and Luminex. A substantial proportion of synovial Tc17 cells co-expressed pro-inflammatory IFN-γ, TNF-α and GM-CSF, whilst IL-21 was co-expressed by less than 20% of Tc17 cells. IL-22 and anti-inflammatory IL-10 were found to be either absent or expressed by only a small proportion of Tc17 cells (Figure 6A, B). IL-17F was found to be co-expressed by only a small proportion of Tc17 cells (Supplementary Figure S6B). The cytokine co-expression profile of synovial Tc17 cells closely resembled the cytokine profile of synovial Th17 cells (Figure 6B, right panel), whilst this profile was not shared by synovial IL-17A- CD8+ or IFN-γ+ CD8+ T cell subsets (Supplementary Figure S6A). Overall, synovial Tc17 cells displayed a polyfunctional cytokine profile with a sizable proportion of cells (median 36%), expressing IL-17A, IFN-γ and TNF-α concomitantly (Figure 6C). This concomitant cytokine production was also observed in the Luminex analysis: sorted IL-17A+ CD8+ T cells from the synovial fluid of patients with PsA (gating strategy in Supplementary Figure S7) produced IL-17A, IFN-γ, TNF-α, GM-CSF and IL-22, with low levels of IL-21 and IL-10 during 24hr culture (Figure 6D).

Finally, we investigated whether Tc17 cells from the inflamed PsA joint express a unique set of markers. For this, we compared the transcriptional profile of synovial Tc17 cells to the profiles of both synovial Tc1 and Th17 cells, to determine which markers are uniquely upregulated in synovial Tc17 cells. Bioinformatic analysis revealed that 15 genes were upregulated in both comparisons, suggesting synovial Tc17 cells have a small unique transcriptional profile in comparison to synovial Tc1 and Th17 cells (Figure 6E). Of these 15 genes, 6 were genes were found to be consistently upregulated in all three patients: *CXCR6*, *NCR3*, *CHN1*, *LINC02195*, *LINC01871* and *GOLIM4*. Since CXCR6 is part of the T_{RM} gene signature (16), we validated CXCR6 at the protein level and found that PsA synovial Tc17 cells indeed expressed CXCR6 at the highest level and contained the highest proportion of CXCR6-expressing cells as compared to their synovial Tc1 and Th17 counterparts (Figure 6F). In addition, levels of CXCL16, the ligand for CXCR6, were significantly increased in PsA synovial fluid vs. paired serum (Figure 6G).

DISCUSSION

We previously described an enrichment of IL-17A+ CD4- (the majority of which were CD8+) T cells in the synovial fluid of patients with PsA compared to the peripheral blood (10). Here we show frequencies of IL-17A+ CD8+ T cells are also increased in the synovial fluid of patients with other types of SpA, and confirm these cells are not increased in RA (10). These findings add to the growing evidence that CD8+ T cells and the IL-17/IL-23 axis are relevant to the immunopathogenesis of PsA/SpA (2). We demonstrate that the vast majority of PsA synovial Tc17 cells are memory cells, indicated by a CD45RA-PD1+HLA-DR+ profile, as well as $\alpha\beta$ TCR bearing, suggesting these cells are MHC class I restricted and antigen-experienced. Previous studies demonstrated oligoclonal expansion of joint-derived bulk T cells in PsA (21-23). Our TCRβ sequencing of synovial Tc17 cells, although limited in sample size, showed that although some T cell clones occupied >5% of the total TCR repertoire, the majority of Tc17 cells, as well as of the bulk memory CD8+ T cells and Tc1 cells, had a polyclonal TCR repertoire. One reason for the differences observed between the previous studies and our own could be patient disease activity. A study investigating the clonality of psoriatic skin-derived CD8+ T cells reported that the TCR repertoire is polyclonal in active disease, whilst in resolved disease only a few dominant clones persist (28). We obtained synovial fluid effusions from actively inflamed joints, which could explain the observed polyclonal repertoire. It would be of interest to investigate if a polyclonal repertoire is also observed in resolved PsA joints, however, this would require a synovial biopsy study design as non-inflamed joints rarely contain sufficient synovial fluid for aspiration.

A notable observation in our analysis was the extensive sharing of T cell clones between the synovial Tc17 and Tc1 populations. Our RNAseq analysis also showed significant overlap in transcriptional profile between Tc17 and Tc1 cells. A possible explanation for these findings is that synovial Tc17 and Tc1 cells may have shared ancestry which raises the question of plasticity between these subsets. Adoptive transfer of *in vitro* generated Tc17 cells into recipient mice showed that Tc17 cells can switch to an IL-17A-negative Tc1 profile (29-31). A direct demonstration of this phenomenon came from an elegant IL-17A fate mapping study using reporter donor

IL-17^{Cre}Rosa26^{eYFP} (13). This study showed that Tc17 cells developed early after allogeneic stem cell transplantation in both lymphoid tissue and graft-versus-host-disease target organs. However, their production of IL-17A was transient whilst IFN-γ production was largely maintained, a process defined by the surrounding cytokine milieu. A similar capacity to transition to IFN-γ production and Th1 phenotype had previously been shown for mouse Th17 cells (32). These fate mapping data, together with our findings on shared TCR and transcriptional profiles and the observation that a high frequency of synovial Tc17 cells express IFN-γ, could suggest that synovial Tc17 cells can transition to a Tc1-like cytokine profile, as was previously suggested for Th17 and Th1 cells in the JIA joint (33). If this is indeed the case, then one implication of this finding would be that the percentage of IL-17A+ CD8+ T cells detected by flow cytometry may underrepresent the contribution that Tc17 cells make or have made to the synovial T cell compartment.

In addition to Tc17/Tc1 overlap, we observed a phenotypic and molecular overlap between Tc17 and Th17 cells in PsA, as evaluated by RNA-seq, RT-qPCR and flow cytometry. Two markers typically expressed by Th17 cells, CCR6 and CD161 (34-36), are co-expressed by a large proportion of synovial Tc17 cells. Furthermore, expression of *IL23R* by synovial Tc17 cells indicates that IL-23 may be involved in generation or maintenance of these cells, whilst elevated *RORC* transcript expression suggests that RORγt may at least play a part in IL-17A regulation in these cells. These data indicate that Tc17 cells may be regulated by similar pathways as Th17 cells.

The relatively limited sample sizes in the TCR and RNA-seq analysis could be considered a limitation of our study. However, given the challenges in obtaining sufficient numbers from small populations of immune cells, this number of samples (n=3) is not uncommon in studies on human tissue-derived cells. In further mitigation, many of our RNA-seq results were independently validated at the protein or RNA level. A further limitation of our work is that we did not have access to synovial tissue samples from patients with PsA. Nonetheless, the work presented here provides an important basis for future studies aimed at comparing the

phenotype and molecular profile of synovial fluid and synovial tissue-derived Tc17 cells.

A key novel finding from our work is that synovial Tc17 cells are part of the tissue-resident memory T cell compartment and express molecules that prevent egress from the inflamed tissue into the blood. T_{RM} cells are rapidly emerging as potential contributors to inflammation in several immune-mediated inflammatory diseases (17, 37). To our knowledge, the data presented here are the first description that Tc17 cells form part of the synovial T_{RM} pool and only the second description of T_{RM}-like cells in the context of human immune-mediated arthritis (11). Our data also show that a high proportion of synovial Tc17 cells express markers typically associated with homing to the skin or gut. This could indicate that synovial Tc17 cells have tropism for these tissues as well as for the synovial compartment and indeed, the presence of Tc17 cells has been described in both the skin and the gut (24, 25, 27). An alternative explanation could be that synovial Tc17 cells expressing these markers have enhanced adherence to the surrounding joint tissue, as both CLA and CD49a ligands (E-selectin and Type IV collagen, respectively) are present in the synovial tissue (38, 39). Integrin β7, which is expressed by a large proportion of synovial Tc17 cells, can form a heterodimer with the T_{RM} cell marker CD103 (αE integrin), which is expressed by Tc17 cells. The product, αEβ7 integrin, exclusively binds E-Cadherin, a structural protein expressed in the synovial joint and fluid of patients with inflammatory arthritis (39, 40). Interaction of synovial Tc17 cells with the surrounding tissue and extracellular matrix in the fluid combined with lack of response to exit cues (e.g. S1P1) could represent one way by which Tc17 cells are retained in the synovial joint and contribute to perpetuation or re-initiation of inflammation. Residence of synovial Tc17 cells in the inflamed tissue may be further enhanced by their high expression of CXCR6, a marker of T_{RM} cells (16), in combination with the increased levels of CXCL16 in the PsA synovial joint. CXCL16 has chemotactic and angiogenic properties and can be produced as a soluble mediator or as a transmembrane-bound chemokine by monocytes, macrophages and dendritic cells. Evidence of increased CXCL16 and CXCR6 expression at the site of inflammation was reported previously in the context of RA and psoriasis, and CXCL16 was shown to enhance recruitment of inflamed tissue-derived CXCR6-expressing T cells (41-43). Furthermore, CXCL16 blockade or CXCR6 deficiency led

to reduced arthritis scores and lower IFN-γ/IL-17 production in an experimental model of arthritis (42, 44). Together, these data suggest that the increased CXCR6 expression on synovial Tc17 cells may contribute to their recruitment and persistence in the inflamed PsA joint.

Functionally, our data indicate that synovial Tc17 cells are polyfunctional and actively secrete several pro-inflammatory cytokines (IFN-γ, TNF-α, GM-CSF, IL-21 and IL-22) in parallel to IL-17A, but little IL-10 or IL-17F. IFN-γ, TNF-α and GM-CSF have all been shown to act synergistically with IL-17A to promote inflammation. In a recent study by Wade et al. polyfunctional CD8+ T cells were also found to be enriched in the PsA synovium, although enrichment was not observed for single cytokine producing T cells, including IL-17A+ CD8+ T cells (45). Concordant with their pro-inflammatory cytokine production, Tc17 cells co-expressed cytolytic molecules granzyme A and granzyme B at comparable levels to synovial Tc1 cells. However, Tc17 cells lacked significant expression of other cytolytic machinery (perforin and LAMP1). Most studies to date in both mice and humans report that Tc17 cells lack cytolytic function (29, 31, 46, 47), although some evidence for cytotoxic function has been reported (24, 48). An interesting alternative interpretation of our data is that the secretion of granzymes is not cytolytic but leads to extracellular matrix degradation or promotion of inflammation (49, 50). Collectively, these data show that Tc17 cells are armed with an array of pro-inflammatory mediators, which could act directly on surrounding cells to promote inflammation in the synovial joint.

In summary, our findings reveal that synovial Tc17 cells from the PsA joint bear hallmarks of tissue resident memory T cells, and express high levels of CXCR6, which may enhance retention of these cells in the inflamed joint. Our analysis of the transcriptional profile and TCR repertoire of these cells highlights several commonalities between Tc17 and Tc1 cells in the PsA joint. Combined with the observed polyfunctional pro-inflammatory mediator production, we hypothesise that Tc17 cells exert heterogenous effector responses which contribute to initiation and persistence of disease in PsA.

AUTHOR CONTRIBUTIONS

KJAS designed and performed the majority of the experiments, analysed data and wrote the manuscript, US sorted cells for RNA-seq and analysed data, MR performed bioinformatic analysis of the RNA-seq data, LED performed and analysed the TCR sequencing and RT-qPCR experiments, SYW and SR processed samples and performed flow cytometry experiments, CH collected clinical samples and performed some of the T_{RM} stains, EC recruited patients, collected samples and provided clinical information, BWK contributed with clinical samples and expertise, to study design and writing of the manuscript, LST supervised the study, contributed to study concept and design, experimental design, interpretation of the data and wrote the manuscript. All authors read and approved the manuscript.

ACKNOWLEDGEMENTS

This study was supported by a King's Health Partners R&D challenge award (R140808), an MRC clinical training fellowship to LD (Ref MR/P018904/1), a King's Health Schools PhD studentship to US funded by a Medical Research Council doctoral training grant, a research grant from Novartis Pharma AG, a Versus Arthritis programme grant (21139) and by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health. The authors would like to thank Sylvine Lalnunhlimi (King's College London) for help in processing some of the patient samples, Celine Trouillet, Yasmin Haque and the BRC Flow Core for their help in flow sorting, and Dr Esperanza Perucha (King's College London) for critical reading of the manuscript.

REFERENCES

1. Stolwijk C, Boonen A, van Tubergen A, and Reveille JD. Epidemiology of spondyloarthritis. *Rheum Dis Clin North Am.* 2012;38(3):441-76.
2. Taams LS, Steel KJA, Srenathan U, Burns LA, and Kirkham BW. IL-17 in the immunopathogenesis of spondyloarthritis. *Nature Reviews Rheumatology.* 2018;14:453-66.
3. Gravallese EM, and Schett G. Effects of the IL-23-IL-17 pathway on bone in spondyloarthritis. *Nat Rev Rheumatol.* 2018;14(11):631-40.
4. Mease PJ, McInnes IB, Kirkham B, Kavanaugh A, Rahman P, van der Heijde D, et al. Secukinumab Inhibition of Interleukin-17A in Patients with Psoriatic Arthritis. *New England Journal of Medicine.* 2015;373(14):1329-39.
5. Baeten D, Sieper J, Braun J, Baraliakos X, Dougados M, Emery P, et al. Secukinumab, an Interleukin-17A Inhibitor, in Ankylosing Spondylitis. *New England Journal of Medicine.* 2015;373(26):2534-48.
6. International Genetics of Ankylosing Spondylitis C. Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci. *Nature Genetics.* 2013;45:730.
7. Bowes J, Budu-Aggrey A, Huffmeier U, Uebe S, Steel K, Hebert HL, et al. Dense genotyping of immune-related susceptibility loci reveals new insights into the genetics of psoriatic arthritis. *Nature Communications.* 2015;6:6046.
8. Winchester R, Minevich G, Steshenko V, Kirby B, Kane D, Greenberg DA, et al. HLA associations reveal genetic heterogeneity in psoriatic arthritis and in the psoriasis phenotype. *Arthritis Rheum.* 2012;64(4):1134-44.
9. Cortes A, Pulit SL, Leo PJ, Pointon JJ, Robinson PC, Weisman MH, et al. Major histocompatibility complex associations of ankylosing spondylitis are complex and involve further epistasis with ERAP1. *Nat Commun.* 2015;6:7146.
10. Menon B, Gullick NJ, Walter GJ, Rajasekhar M, Garrood T, Evans HG, et al. IL-17+CD8+ T-cells are enriched in the joints of patients with psoriatic arthritis and correlate with disease activity and joint damage progression. *Arthritis Rheumatol.* 2014;66:1272-81.
11. Petrelli A, Mijnheer G, van Konijnenburg DPH, van der Wal MM, Giovannone B, Mocholi E, et al. PD-1+CD8+ T cells are clonally expanding effectors in human chronic inflammation. *The Journal of Clinical Investigation.* 2018;128(10):4669-81.
12. Srenathan U, Steel K, and Taams LS. IL-17+ CD8+ T cells: Differentiation, phenotype and role in inflammatory disease. *Immunology Letters.* 2016;178:20-6.
13. Gartlan KH, Markey KA, Varelias A, Bunting MD, Koyama M, Kuns RD, et al. Tc17 cells are a proinflammatory, plastic lineage of pathogenic CD8+ T cells that induce GVHD without antileukemic effects. *Blood.* 2015;126(13):1609-20.
14. Mielke LA, Liao Y, Clemens EB, Firth MA, Duckworth B, Huang Q, et al. TCF-1 limits the formation of Tc17 cells via repression of the MAF-RORgammat axis. *J Exp Med.* 2019.
15. Sathaliyawala T, Kubota M, Yudanin N, Turner D, Camp P, Thome Joseph JC, et al. Distribution and Compartmentalization of Human Circulating and Tissue-Resident Memory T Cell Subsets. *Immunity.* 2013;38(1):187-97.

16. Kumar BV, Ma W, Miron M, Granot T, Guyer RS, Carpenter DJ, et al. Human Tissue-Resident Memory T Cells Are Defined by Core Transcriptional and Functional Signatures in Lymphoid and Mucosal Sites. *Cell Rep.* 2017;20(12):2921-34.
17. Masopust D, and Soerens AG. Tissue-Resident T Cells and Other Resident Leukocytes. *Annu Rev Immunol.* 2019.
18. Clark RA. Resident memory T cells in human health and disease. *Sci Transl Med.* 2015;7(269):269rv1.
19. Taylor W, Gladman D, Helliwell P, Marchesoni A, Mease P, and Mielants H. Classification criteria for psoriatic arthritis: Development of new criteria from a large international study. *Arthritis & Rheumatism.* 2006;54(8):2665-73.
20. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, et al. 2010 Rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis & Rheumatism.* 2010;62(9):2569-81.
21. Tassiulas I, Duncan SR, Centola M, Theofilopoulos AN, and Boumpas DT. Clonal characteristics of T cell infiltrates in skin and synovium of patients with psoriatic arthritis. *Hum Immunol.* 1999;60(6):479-91.
22. Costello PJ, Winchester RJ, Curran SA, Peterson KS, Kane DJ, Bresnihan B, et al. Psoriatic arthritis joint fluids are characterized by CD8 and CD4 T cell clonal expansions appear antigen driven. *J Immunol.* 2001;166(4):2878-86.
23. Curran SA, FitzGerald OM, Costello PJ, Selby JM, Kane DJ, Bresnihan B, et al. Nucleotide sequencing of psoriatic arthritis tissue before and during methotrexate administration reveals a complex inflammatory T cell infiltrate with very few clones exhibiting features that suggest they drive the inflammatory process by recognizing autoantigens. *J Immunol.* 2004;172(3):1935-44.
24. Ortega C, FernÃ¡ndez-A S, Carrillo JM, Romero P, Molina IJ, Moreno JC, et al. IL-17-producing CD8+ T lymphocytes from psoriasis skin plaques are cytotoxic effector cells that secrete Th17-related cytokines. *Journal of Leukocyte Biology.* 2009;86(2):435-43.
25. Res PCM, Piskin G, de Boer OJ, van der Loos CM, Teeling P, Bos JD, et al. Overrepresentation of IL-17A and IL-22 producing CD8 T cells in lesional skin suggests their involvement in the pathogenesis of psoriasis. *PLoS ONE.* 2010;5(11):e14108.
26. Hijnen D, Knol EF, Gent YY, Giovannone B, J P Beijn S, Kupper TS, et al. CD8+ T cells in the lesional skin of atopic dermatitis and psoriasis patients are an important source of IFN-g, IL-13, IL-17, and IL-22. *J Invest Dermatol.* 2013;133(4):973-9.
27. Tom MR, Li J, Ueno A, Fort Gasia M, Chan R, Hung DY, et al. Novel CD8+ T-Cell Subsets Demonstrating Plasticity in Patients with Inflammatory Bowel Disease. *Inflamm Bowel Dis.* 2016;22(7):1596-608.
28. Matos TR, O'Malley JT, Lowry EL, Hamm D, Kirsch IR, Robins HS, et al. Clinically resolved psoriatic lesions contain psoriasis-specific IL-17–producing αβ T cell clones. *The Journal of Clinical Investigation.* 2017;127(11):4031-41.
29. Yen H-R, Harris TJ, Wada S, Grosso JF, Getnet D, Goldberg MV, et al. Tc17 CD8 T Cells: Functional Plasticity and Subset Diversity. *The Journal of Immunology.* 2009;183(11):7161-8.

30. Hinrichs CS, Kaiser A, Paulos CM, Cassard L, Sanchez-Perez L, Heemskerk B, et al. Type 17 CD8+ T cells display enhanced antitumor immunity. *Blood.* 2009;114(3):596-9.
31. Flores-Santibanez F, Cuadra B, Fernandez D, Rosemblatt MV, Nunez S, Cruz P, et al. In Vitro-Generated Tc17 Cells Present a Memory Phenotype and Serve As a Reservoir of Tc1 Cells In Vivo. *Front Immunol.* 2018;9:209.
32. Hirota K, Duarte JH, Veldhoen M, Hornsby E, Li Y, Cua DJ, et al. Fate mapping of IL-17-producing T cells in inflammatory responses. *Nat Immunol.* 2011;12(3):255-63.
33. Nistala K, Adams S, Cambrook H, Ursu S, Olivito B, de Jager W, et al. Th17 plasticity in human autoimmune arthritis is driven by the inflammatory environment. *Proceedings of the National Academy of Sciences.* 2010;107(33):14751-6.
34. Maggi L, Santarlasci V, Capone M, Peired A, Frosali F, Crome SQ, et al. CD161 is a marker of all human IL-17-producing T-cell subsets and is induced by RORC. *European Journal of Immunology.* 2010;40(8):2174-81.
35. Cosmi L, Cimaz R, Maggi L, Santarlasci V, Capone M, Borriello F, et al. CD4+CD161+ T cells showing transient nature of the Th17 phenotype are present in the synovial fluid from patients with juvenile idiopathic arthritis. *Arthritis & Rheumatism.* 2011:n/a-n/a.
36. Annunziato F, Cosmi L, Liotta F, Maggi E, and Romagnani S. Defining the human T helper 17 cell phenotype. *Trends in immunology.* 2012;33(10):505-12.
37. Park CO, and Kupper TS. The emerging role of resident memory T cells in protective immunity and inflammatory disease. *Nat Med.* 2015;21(7):688-97.
38. Klimiuk PA, Sierakowski S, Latosiewicz R, Cylwik JP, Cylwik B, Skowronski J, et al. Soluble adhesion molecules (ICAM-1, VCAM-1, and E-selectin) and vascular endothelial growth factor (VEGF) in patients with distinct variants of rheumatoid synovitis. *Ann Rheum Dis.* 2002;61(9):804-9.
39. Steenvoorden MM, Tolboom TC, van der Pluijm G, Lowik C, Visser CP, DeGroot J, et al. Transition of healthy to diseased synovial tissue in rheumatoid arthritis is associated with gain of mesenchymal/fibrotic characteristics. *Arthritis Res Ther.* 2006;8(6):R165.
40. Melis L, Van Praet L, Pircher H, Venken K, and Elewaut D. Senescence marker killer cell lectin-like receptor G1 (KLRG1) contributes to TNF-alpha production by interaction with its soluble E-cadherin ligand in chronically inflamed joints. *Ann Rheum Dis.* 2014;73(6):1223-31.
41. Van Der Voort R, Van Lieshout AWT, Toonen LWJ, Slöetjes AW, Van Den Berg WB, Figdor CG, et al. Elevated CXCL16 expression by synovial macrophages recruits memory T cells into rheumatoid joints. *Arthritis Rheum.* 2005;52(5):1381-91.
42. Nanki T, Shimaoka T, Hayashida K, Taniguchi K, Yonehara S, and Miyasaka N. Pathogenic role of the CXCL16-CXCR6 pathway in rheumatoid arthritis. *Arthritis Rheum.* 2005;52(10):3004-14.
43. Gunther C, Carballido-Perrig N, Kaesler S, Carballido JM, and Biedermann T. CXCL16 and CXCR6 are upregulated in psoriasis and mediate cutaneous recruitment of human CD8+ T cells. *J Invest Dermatol.* 2012;132(3 Pt 1):626-34.
44. Slauenwhite D, Gebremeskel S, Doucette CD, Hoskin DW, and Johnston B. Regulation of cytokine polarization and T cell recruitment to inflamed paws in

mouse collagen-induced arthritis by the chemokine receptor CXCR6. *Arthritis Rheumatol.* 2014;66(11):3001-12.
45. Wade SM, Canavan M, McGarry T, Low C, Wade SC, Mullan RH, et al. Association of synovial tissue polyfunctional T-cells with DAPSA in psoriatic arthritis. *Annals of the Rheumatic Diseases.* 2019:annrheumdis-2018-214138.
46. Hamada H, Garcia-Hernandez Mde L, Reome JB, Misra SK, Strutt TM, McKinstry KK, et al. Tc17, a unique subset of CD8 T cells that can protect against lethal influenza challenge. *J Immunol.* 2009;182(6):3469-81.
47. Cheuk S, Schlums H, Gallais Sérézal I, Martini E, Chiang SC, Marquardt N, et al. CD49a Expression Defines Tissue-Resident CD8+ T Cells Poised for Cytotoxic Function in Human Skin. *Immunity.* 2017;46(2):287-300.
48. Yeh N, Glosson NL, Wang N, Guindon L, McKinley C, Hamada H, et al. Tc17 cells are capable of mediating immunity to vaccinia virus by acquisition of a cytotoxic phenotype. *J Immunol.* 2010;185(4):2089-98.
49. Wensink AC, Hack CE, and Bovenschen N. Granzymes regulate proinflammatory cytokine responses. *J Immunol.* 2015;194(2):491-7.
50. Santiago L, Menaa C, Arias M, Martin P, Jaime-Sanchez P, Metkar S, et al. Granzyme A Contributes to Inflammatory Arthritis in Mice Through Stimulation of Osteoclastogenesis. *Arthritis Rheumatol.* 2017;69(2):320-34.

FIGURE LEGENDS

Figure 1. IL-17A+ TCRαβ+ CD8+ T cells are enriched in the synovial fluid of patients with SpA. A) Representative staining and B) cumulative data depicting frequencies of IL-17A+ cells within CD3+ CD8+ T cells in paired PBMC and SFMC from patients with PsA (left, n=18), other peripheral SpA (centre, n=14) or RA (right, n=6) after 3 hours stimulation in the presence of PMA, ionomycin and Golgistop. Data were analysed using Wilcoxon's matched pairs signed rank test. C) Representative staining and D) cumulative data (median + IQR) showing the frequency of αβ TCR (n=8), γδ TCR (n=9), CD56 (n=7) and Vα7.2 (n=6) expressing IL-17A+ CD8+ T cells from PsA SF (stimulated as before). E) Representative staining and F) frequencies of CD45RA and/or CD27 expressing cells (median + IQR) within IL-17A+ CD8+ T cells from the SFMC of patients with PsA (n=6) (stimulated as before). G) Representative staining and H) cumulative data (median + IQR) showing the frequencies of PD-1 and HLA-DR expressing IL-17A+ CD8+ T cells from PsA patients (n=6).

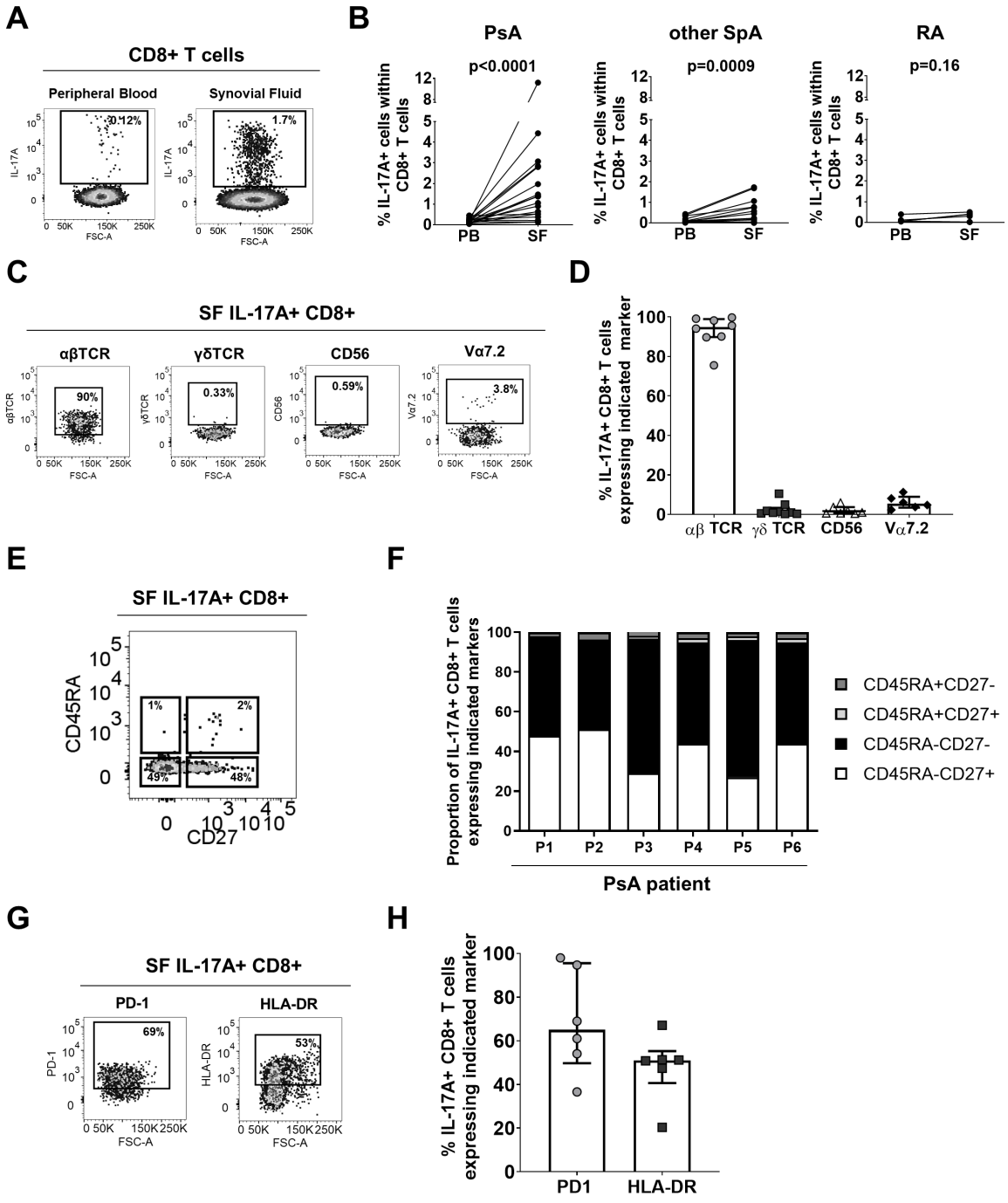
Figure 2. Synovial Tc17 cells have a diverse TCR repertoire A) Representative pie charts showing the frequency of all TCRβ sequences in bulk CD8+ memory, IL-17A+ CD8+ and IL-17A- IFN-γ+ CD8+ T cells. B) Clonality score (defined as 1-Pielou's evenness) of bulk CD8+ memory, IL-17A+ CD8+ and IL-17A- IFN-γ+ CD8+ T cells from PsA synovial fluid samples (n=3). C) Individual dot plots and D) clonal overlap defined as Morisita's index between IL-17A+ CD8+ and IL-17A- IFN-γ+ CD8+ T cells.

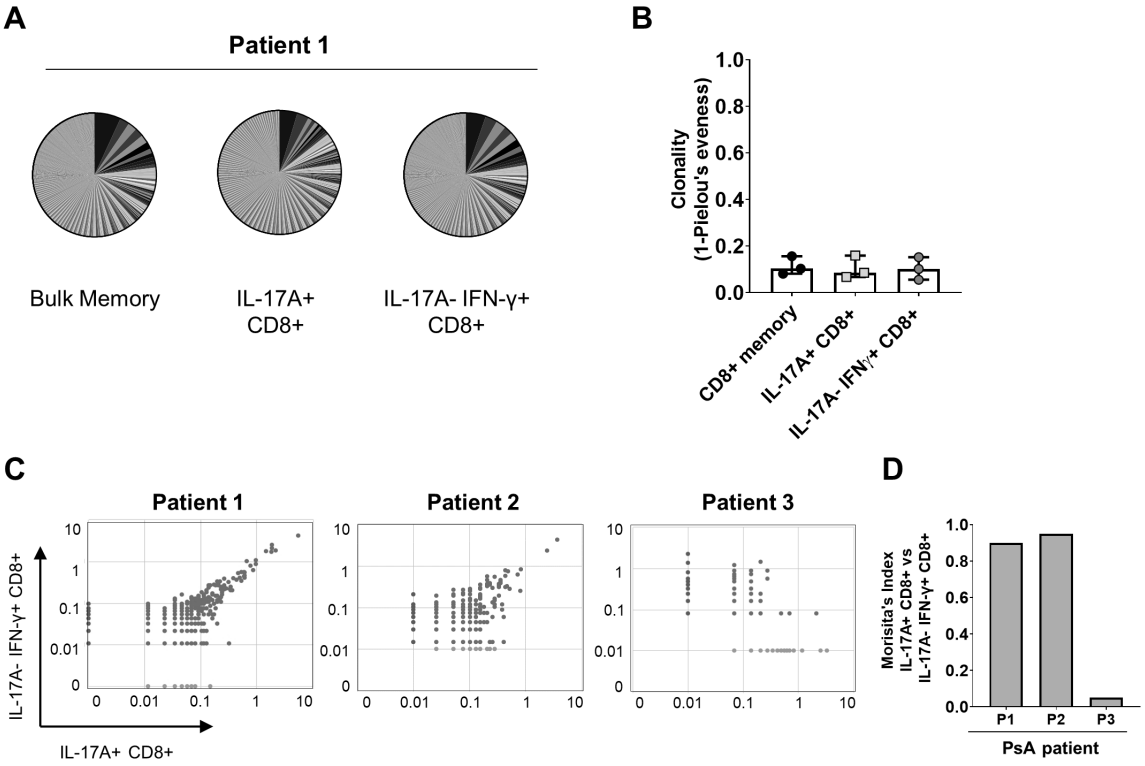
Figure 3. Transcriptional profile of synovial Tc17 cells compared to Tc1 and Th17 cells. A, B) Principal component analysis of the transcriptome of IL-17A+ CD8+, IFN-γ+ CD8+ and IL-17A+ CD4+ T cells from the synovial fluid of PsA patients (n=3), colour-indicated by cell type (A) or patient (B). (C, D) MA plots and (E, F) selected gene expression profiles shown as average gene expression values (FPKM) of synovial IL-17A+ CD8+ compared to IFN-γ+ CD8+ T cells or CD4+ IL-17A+ T cells (n=3). In C, D: grey points = adjusted p value>0.01, blue points = adjusted p value <0.01, red points = adjusted p value <0.001.

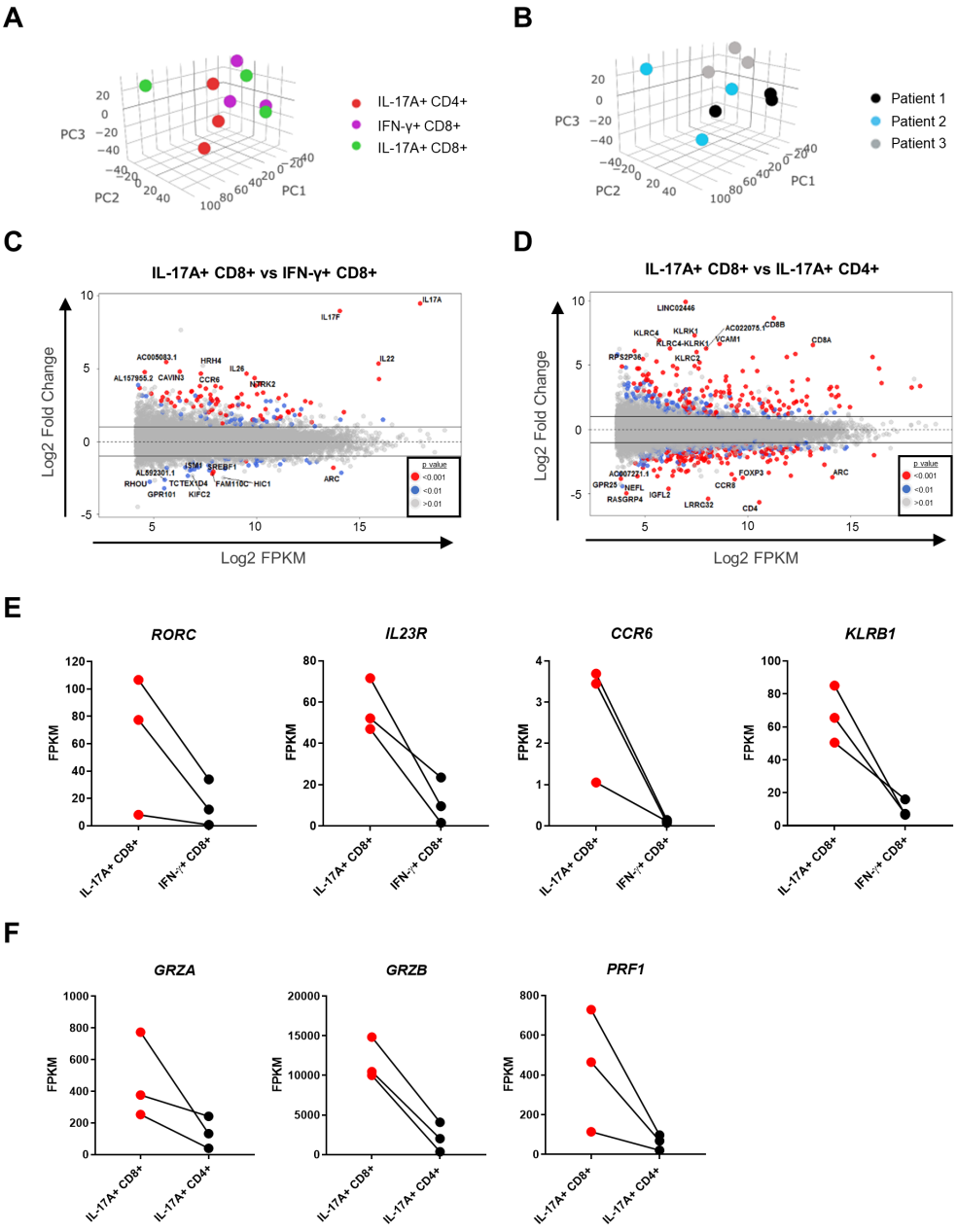
Figure 4. Synovial Tc17 cells have functional hallmarks of Th17 and Tc1 cells. A) Representative staining and B) cumulative data showing the frequency of IL-17A+ CD8+, IL-17A+ CD4+, IL-17A- CD8+ and IFN-γ+ CD8+ T cells from SFMC of PsA/SpA (PsA = colour symbols; other SpA = white symbols) expressing CCR6 (n=7-9) and CD161 (n=7-9) (median + IQR). C) Representative ViSNE plot showing concomitant expression of IL-17A, CCR6 and CD161 within CD8+ T cells from the SFMC of PsA patients (representative of n=7). D) Gene expression levels of *RORC* and *IL23R* normalised to the average values for *18S* and *UBC* in sorted IL-17A+ CD8+, IL-17A- CD8+ and IL-17A+ CD4+ T cells from PsA SFMC (n=4). E) Representative staining and F) cumulative data (median + IQR) showing the frequencies of granzyme A (n=4), granzyme B (n=9), LAMP1 (n=6) and perforin (n=6) expressing cells within IL-17A+ CD8+, IFN-γ+ CD8+ or IL-17A+ CD4+ T-cells from SFMC of PsA patients. Analysed using Friedman multiple comparisons test

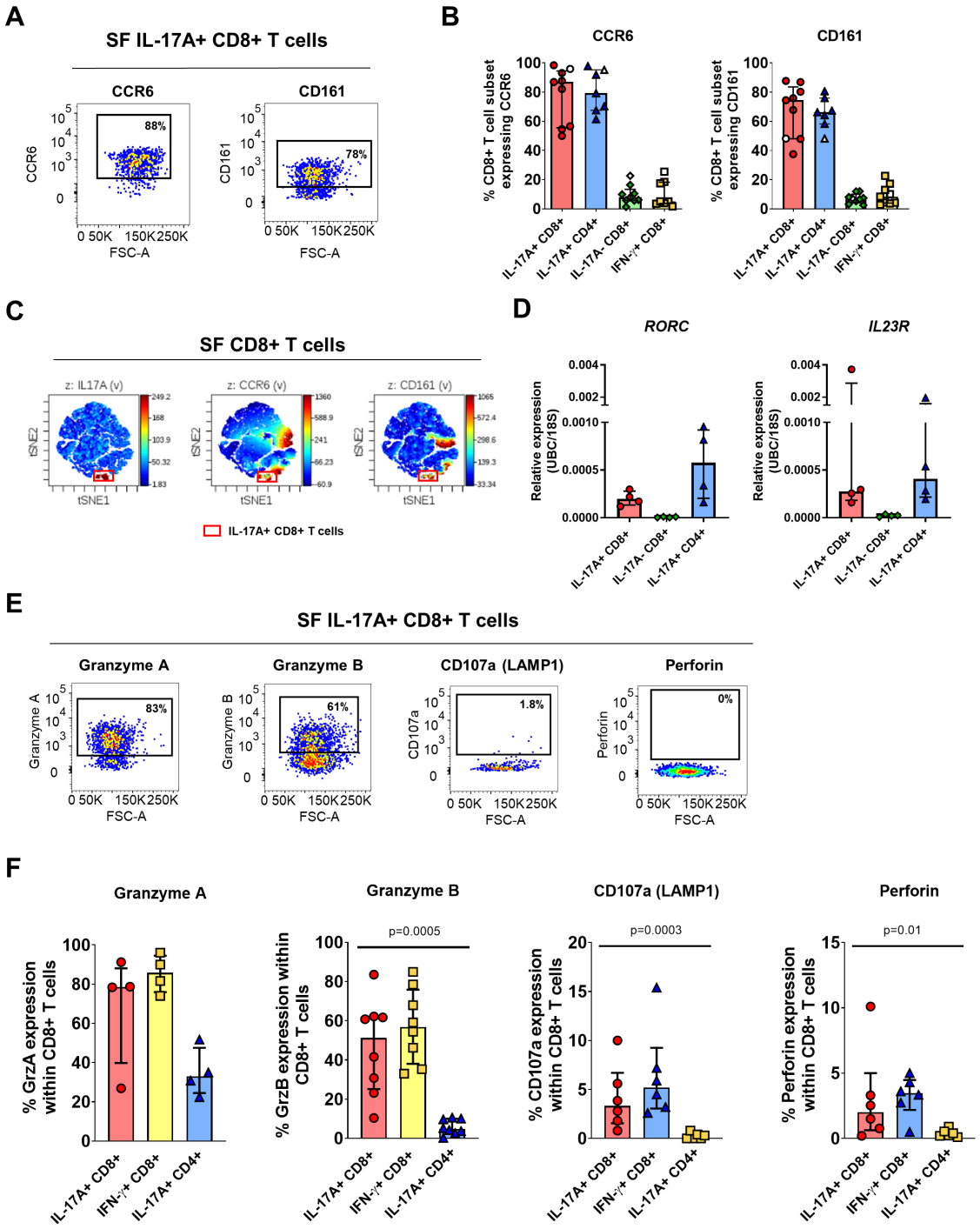
Figure 5. Synovial Tc17 form part of the synovial tissue-resident T cell compartment. A) Selected T_{RM}-associated gene expression profiles of sorted synovial IL-17A+ CD8+ T cells compared to CD4+ IL-17A+ T cells (n=3). B) Representative staining and C) cumulative data (median + IQR) showing the frequencies of IL-17A+ CD8+ T cells from SFMC of patients with PsA expressing CD103/αE integrin (n=12), integrin β7 (n=7), CD49a/VLA1 (n=9) or CLA (n=12), after 3 hours stimulation with PMA, ionomycin and Golgistop. D) Representative staining and E) frequencies of CD69+CD103+, CD69+CD103- and CD69-CD103+ T_{RM} cells (median + IQR) within synovial CD3+CD8+ (n=12) and CD3+CD4+ T cells (n=12). F-H) Cells from the synovial fluid of patients with PsA (n=3) were sorted into CD69+CD103+, CD69+CD103-, CD69-CD103+ and CD69-CD103- CD8+ T cells. Sorted subsets were stimulated with PMA and ionomycin in the presence of Golgistop for 3 hours. F, G) Representative staining showing IL-17A (F) and IFN-γ (G) expression within the 4 sorted subsets. H) Proportion of IL-17A+ (left panel, n=3) or IFN-γ+ (right panel, n=2) cells in each T_{RM} subset, as a fraction of total IL-17A or IFN-γ expressing cells.

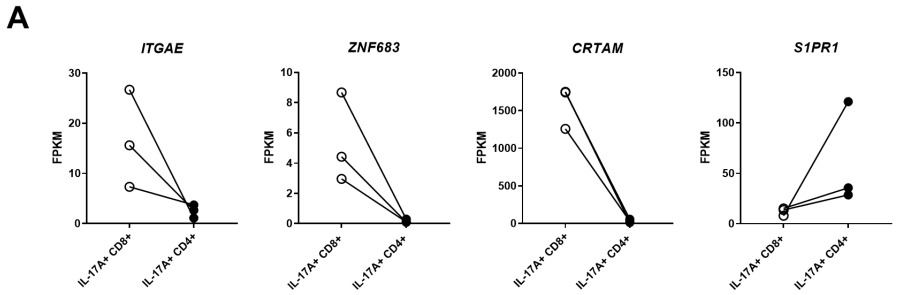
Figure 6. Synovial Tc17 cells are polyfunctional pro-inflammatory cells characterised by high expression of CXCR6 A) Representative and B) cumulative data (median+IQR) showing IFN-γ (n=12), TNF-α (n=12), GM-CSF (n=9), IL-21 (n=6), IL-22 (n=6) and IL-10 (n=8) co-expressing cells within IL-17A+ CD8+ (left panel) and IL-17A+ CD4+ (right panel) T cells from PsA (closed symbols) or other SpA (open symbols) SFMC. (C) Proportion of IL-17A+ CD8+ T cells expressing IL-17A alone, IL-17A plus TNF-α or IFN-γ, or IL-17A plus TNF-α and IFN-γ (median+IQR, n=7). D) IL-17A, IFN-γ, TNF-α, GM-CSF, IL-21, IL-22 and IL-10 secretion by sorted synovial IL-17A+ CD8+ T cells (n=4) E) Venn diagram showing number of significantly upregulated genes ($p<0.01$) in IL-17A+ CD8+ T cells vs. (left) IFN-γ+ CD8+ or (right) IL-17A+ CD4+ T cells; heatmap indicates consistently upregulated genes in all patients. F) Representative dot plots/histograms and cumulative data (n=6) showing the percentage of CXCR6+ cells (left panels), and CXCR6 expression levels (right panels) in PsA synovial IL-17A+ CD8+ T cells, IFN-γ+ CD8+ T cells and IL-17A+ CD4+ T cells (Friedman multiple comparisons test). G) CXCL16 levels in paired PsA serum and synovial fluid samples (n=12) as measured by ELISA (Wilcoxon's matched pairs signed rank test).

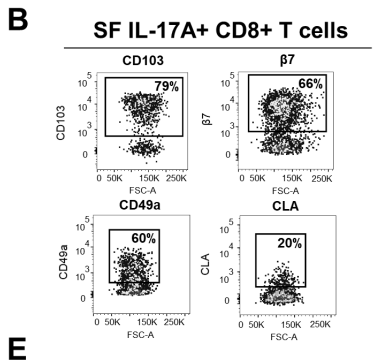

A
CD8+ T cells
Peripheral Blood
Synovial Fluid
0.12%
1.7%
IL-17A
FSC-A
B
PsA
other SpA
RA
p<0.0001
p=0.0009
p=0.16
% IL-17A+ cells within CD8+ T cells
PB
SF
C
SF IL-17A+ CD8+
αβTCR
γδTCR
CD56
Vα7.2
90%
0.33%
0.59%
3.8%
D
% IL-17A+ CD8+ T cells expressing indicated marker
αβ TCR
γδ TCR
CD56
Vα7.2
E
SF IL-17A+ CD8+
CD45RA
CD27
1%
2%
49%
48%
F
Proportion of IL-17A+ CD8+ T cells expressing indicated markers
CD45RA+CD27-
CD45RA+CD27+
CD45RA-CD27-
CD45RA-CD27+
P1
P2
P3
P4
P5
P6
PsA patient
G
SF IL-17A+ CD8+
PD-1
HLA-DR
69%
53%
H
% IL-17A+ CD8+ T cells expressing indicated marker
PD1
HLA-DR

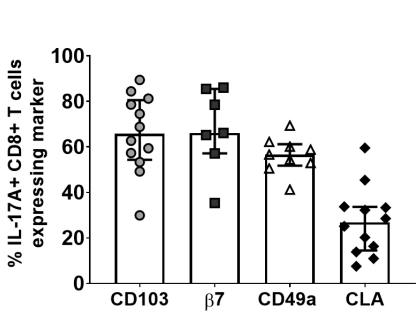

A
Patient 1
Bulk Memory
IL-17A+ CD8+
IL-17A- IFN-γ+ CD8+
B
Clonality (1-Pielou's eveness)
1.0
0.8
0.6
0.4
0.2
0.0
CD8+ memory
IL-17A+ CD8+
IL-17A- IFNγ+ CD8+
C
Patient 1
Patient 2
Patient 3
IL-17A- IFN-γ+ CD8+
IL-17A+ CD8+
10
1
0.1
0.01
0
0.01
0.1
1
10
D
Morisita's Index IL-17A+ CD8+ vs IL-17A- IFN-γ+ CD8+
P1
P2
P3
PsA patient

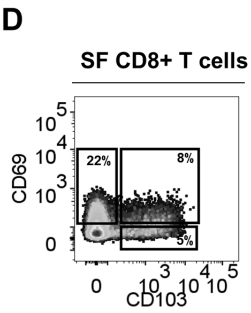

A
PC3
PC2
PC1
IL-17A+ CD4+
IFN-γ+ CD8+
IL-17A+ CD8+
B
PC3
PC2
PC1
Patient 1
Patient 2
Patient 3
C
IL-17A+ CD8+ vs IFN-γ+ CD8+
Log2 Fold Change
Log2 FPKM
p value
<0.001
<0.01
>0.01
D
IL-17A+ CD8+ vs IL-17A+ CD4+
Log2 Fold Change
Log2 FPKM
p value
<0.001
<0.01
>0.01
E
RORC
IL23R
CCR6
KLRB1
FPKM
IL-17A+ CD8+
IFN-γ+ CD8+
F
GRZA
GRZB
PRF1
FPKM
IL-17A+ CD8+
IL-17A+ CD4+

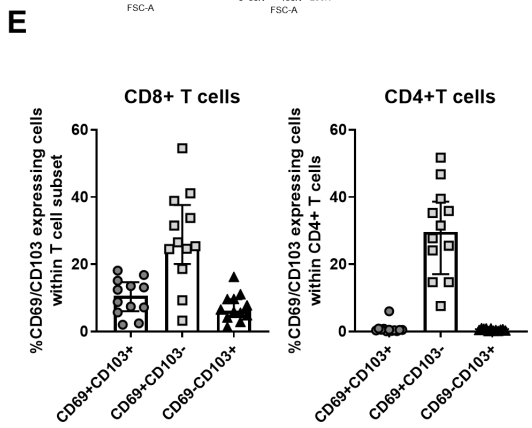

A
SF IL-17A+ CD8+ T cells
CCR6
CD161
88%
78%
CCR6
CD161
FSC-A
B
CCR6
CD161
% CD8+ T cell subset expressing CCR6
% CD8+ T cell subset expressing CD161
IL-17A+ CD8+
IL-17A+ CD4+
IL-17A- CD8+
IFN-/+ CD8+
C
SF CD8+ T cells
z: IL17A (v)
z: CCR6 (v)
z: CD161 (v)
tSNE1
tSNE2
IL-17A+ CD8+ T cells
D
RORC
IL23R
Relative expression (UBC/18S)
IL-17A+ CD8+
IL-17A- CD8+
IL-17A+ CD4+
E
SF IL-17A+ CD8+ T cells
Granzyme A
Granzyme B
CD107a (LAMP1)
Perforin
83%
61%
1.8%
0%
FSC-A
F
Granzyme A
Granzyme B
CD107a (LAMP1)
Perforin
% GrzA expression within CD8+ T cells
% GrzB expression within CD8+ T cells
% CD107a expression within CD8+ T cells
% Perforin expression within CD8+ T cells
p=0.0005
p=0.0003
p=0.01
IL-17A+ CD8+
IFN-/+ CD8+
IL-17A+ CD4+

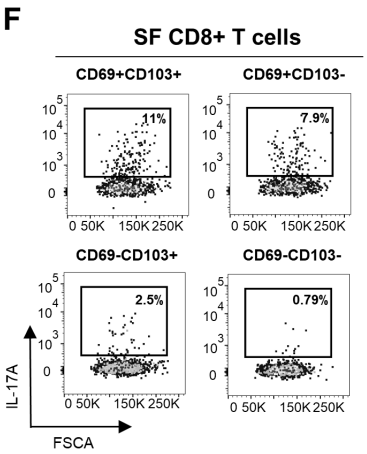

A
ITGAE
ZNF683
CRTAM
S1PR1
FPKM
IL-17A+ CD8+
IL-17A+ CD4+

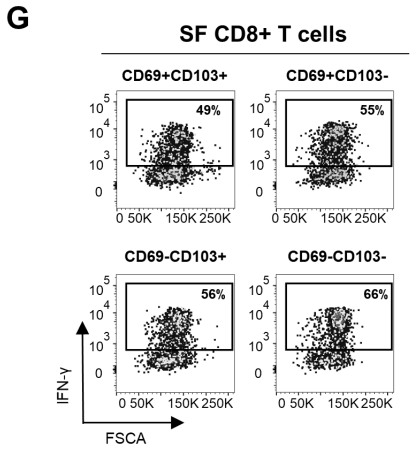

B
SF IL-17A+ CD8+ T cells
CD103
β7
CD49a
CLA
79%
66%
60%
20%
FSC-A

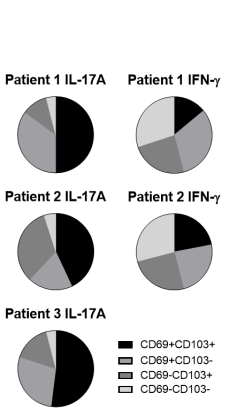

C
% IL-17A+ CD8+ T cells expressing marker
CD103
β7
CD49a
CLA

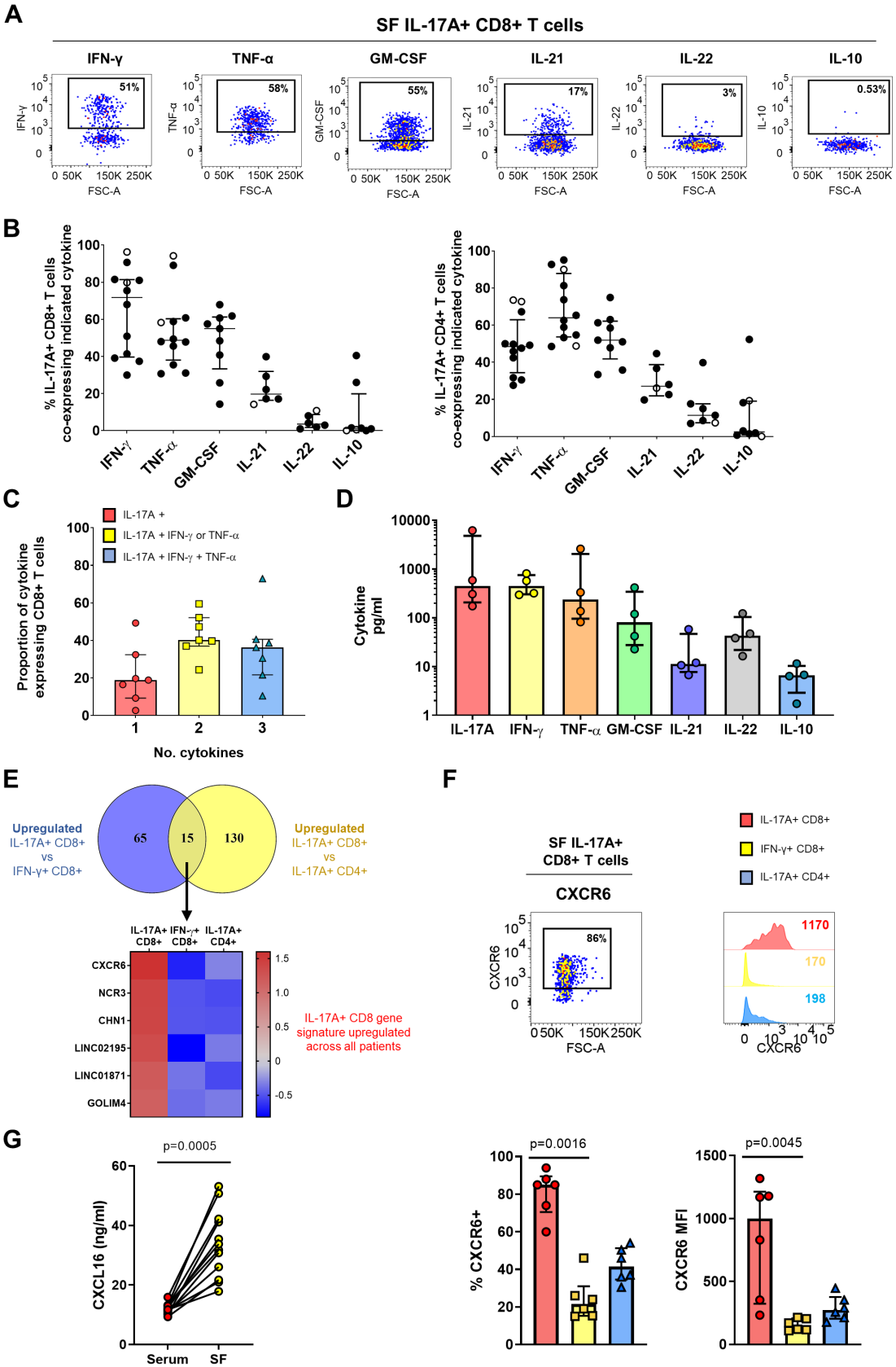

D
SF CD8+ T cells
CD69
CD103
22%
8%
5%


E
CD8+ T cells
CD4+T cells
%CD69/CD103 expressing cells within T cell subset
%CD69/CD103 expressing cells within CD4+ T cells
CD69+CD103+
CD69+CD103-
CD69-CD103+


F
SF CD8+ T cells
CD69+CD103+
CD69+CD103-
CD69-CD103+
CD69-CD103-
11%
7.9%
2.5%
0.79%
IL-17A
FSCA


G
SF CD8+ T cells
CD69+CD103+
CD69+CD103-
CD69-CD103+
CD69-CD103-
49%
55%
56%
66%
IFN-γ
FSCA


H
Patient 1 IL-17A
Patient 1 IFN-γ
Patient 2 IL-17A
Patient 2 IFN-γ
Patient 3 IL-17A
CD69+CD103+
CD69+CD103-
CD69-CD103+
CD69-CD103-


A
SF IL-17A+ CD8+ T cells
IFN-γ
TNF-α
GM-CSF
IL-21
IL-22
IL-10
51%
58%
55%
17%
3%
0.53%
FSC-A
B
% IL-17A+ CD8+ T cells co-expressing indicated cytokine
% IL-17A+ CD4+ T cells co-expressing indicated cytokine
C
IL-17A +
IL-17A + IFN-γ or TNF-α
IL-17A + IFN-γ + TNF-α
Proportion of cytokine expressing CD8+ T cells
No. cytokines
D
Cytokine pg/ml
IL-17A
IFN-γ
TNF-α
GM-CSF
IL-21
IL-22
IL-10
E
Upregulated IL-17A+ CD8+ vs IFN-γ+ CD8+
Upregulated IL-17A+ CD8+ vs IL-17A+ CD4+
65
15
130
IL-17A+ CD8+
IFN-γ+ CD8+
IL-17A+ CD4+
CXCR6
NCR3
CHN1
LINC02195
LINC01871
GOLIM4
IL-17A+ CD8 gene signature upregulated across all patients
F
SF IL-17A+ CD8+ T cells
CXCR6
86%
IL-17A+ CD8+
IFN-γ+ CD8+
IL-17A+ CD4+
1170
170
198
G
p=0.0005
CXCL16 (ng/ml)
Serum
SF
p=0.0016
% CXCR6+
p=0.0045
CXCR6 MFI